Plant Cell Reports (1986) 5:427–429

Plant Cell Reports

Regulation of product synthesis in cell cultures of *Catharanthus roseus*. Effect of culture temperature

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Received June 13, 1986 – Communicated by W. Barz

Abstract

A three year old, alkaloid producing cell line of Catharanthus roseus, maintained at 25°C, was grown on 2% sucrose at various temperatures from 10° to 45°C. Growth rates were maximal at 35°C but declined rapidly above 35°C and below 25°C. Maximum serpentine yields reached a peak at between 20°C and 25°C and fell sharply above and below these temperatures, while ajmalicine showed a sharp peak of accumulation at 20°C. The variable serpentine/ajmalicine ratio at different growth temperatures suggests that lower temperatures may favour ajmalicine accumulation. Both the growth rate and the rate of alkaloid accumulation at 25°C were therefore sensitive to small changes in average culture temperature.

Introduction

Studies of the growth of plant cells in culture have shown that the optimum temperature for growth is generally within the range 25-30°C, although this may vary considerably between different species (Kato et al., 1976; Tulecke, 1960; Matsumoto, 1972; Rose, 1975). Little has been reported however on the effects of culture temperature on secondary metabolite production by cultured cells. Lockwood (1984) showed that temperature shock had little effect on the pattern of alkaloids accumulated by four Papaver species in culture. Courtois and Guern (1980) have previously studied the effect of temperature on growth and indole alkaloid accumulation in C. roseus cultures and found a ten-fold increase in cellular alkaloids at 16°C compared to a temperature of 27°C which gave maximal growth rates. However in terms of alkaloid productivity (amount of product produced l^{-1} d^{-1}) no net increase in alkaloid yield at low temperatures was observed, simply an increase in alkaloid content of the cells. Between 20° and 27°C all cells contributed to the growth of the cell population but at 16°C fewer cells contained the same amount of product. Since the alkaloid induction media used by these workers resulted in much lower alkaloid yields than are possible with C. roseus cells cultured at 25°C in the absence of 2,4-D (Morris, 1986a), it is unclear whether low temperatures stimulate alkaloid accumulation in high yielding cultures.

We have recently shown (Morris, 1986b) that the yield of alkaloids in cell lines of C. roseus grown for several years on a growth and alkaloid production medium, showed seasonal variations. As one of the major variables which must be controlled in the long term maintenance of cell suspension cultures is temperature, the sensitivity of growth and alkaloid production to culture temperature was of particular interest. In the present communication the effect of growth temperature on biomass and alkaloid accumulation in a cell culture of C. roseus capable of high levels of alkaloid biosynthesis is reported.

Materials and Methods

Cell Culture

Initiation and maintenance of the stock cell line of C. roseus (cell line C87) has been described previously (Morris, 1986a). Stock cell cultures were routinely grown on M&S medium (Murashige and Skoog, 1962) with 2% sucrose, 1 mg l^{-1} NAA and 0.1 mg l^{-1} kinetin, pH 5.8 (M3) at 25 ± 1°C in continuous diffuse light. Cells were cultured in 250ml Erlenmeyer flasks containing 100ml medium and were shaken at 150 rpm.

Cells were subcultured every 14 days to fresh medium at a dilution ratio of 1:5.

The C87 cell line used in this work was three years old. The effect of temperature on growth was studied by transferring 14 day old stock cells to fresh medium and culturing at various temperatures in the dark. Growth and alkaloid content of the cells was followed throughout the culture period by sampling from triplicate flasks. Sampling continued until cell viability (FDA method) fell below 50%. The specific growth rate (μ) was calculated over the whole period of fresh wt or dry weight increase and therefore represents an average growth rate over the whole growth period.

Alkaloid analysis

Alkaloids were extracted and analysed as described previously (Morris et al., 1985). Alkaloid fractions from methanol extracts of freeze-dried cells were prepared by ion-pair chromatography using C18 Sep-Pak cartridges (Waters Ass.) and then analysed by reverse-phase, ion-pair HPLC on a μBondapack C18 radial compression cartridge with MeOH: H_2O: n-heptane sulphonate as the eluting solvent. Alkaloids were further identified by TLC.

Results and Discussion

Growth and alkaloid accumulation by the cell line C87 cultured on M_3 medium at temperatures between 10° and 45°C are shown in Figure 1. The growth rate was found to be greatest at 35°C with doubling times in the order of 20 hours at this temperature. No growth occurred at 10°C but cells remained viable for up to 40 days, while at 45°C cell death occurred within 2-5 days. Maximum biomass

yields (both fresh wt and dry wt) occurred at 25°C indicating lower carbon convertion ratios at higher temperatures. Maximum serpentine yields in terms of both g dry wt^{-1} and l^{-1} as well as the maximal rate of serpentine accumulation also occurred at 25°C while the optimal temperature for accumulation of ajmalicine was 20°C. Lower culture temperatures appear therefore to favour higher levels of ajmalicine accumulation.

We have shown previously (Morris, 1986a) that at 25°C serpentine and ajmalicine have different production kinetics in this cell line. Ajmalicine peaked during the culture cycle and was absent from stationary phase cultures while serpentine accumulated throughout the growth cycle. It should be noted therefore that in Figure 1 the maximum levels of serpentine and ajmalicine occurred at different times during the culture period at all temperatures (Fig. 2).

It is evident that alkaloid accumulation is restricted to a narrow temperature range (20-25°C) and that low temperatures while reducing growth rates are as detrimental to alkaloid accumulation as higher temperatures which give maximum growth rates.

Thus one of the factors which may give rise to apparently highly variable alkaloid yielding cell cultures of C. roseus (Morris, 1986b) may be small seasonal variations in the average growth temperatures.

Contrary to the results of Courtois and Guern (1980) low temperatures were found to inhibit alkaloid accumulation. However cell cultures of C. roseus capable of accumulating serpentine and ajmalicine at 25°C to levels ten times the maximum alkaloid levels found in the cell cultures of Courtois and Guern at 16°C were used in this study. These results suggest therefore that 2,4-D inhibition may be reduced by low temperatures and that this may be responsible for the observed increase in serpentine and ajmalicine accumulation in cultures growing in the presence of 2,4-D at low temperatures. In fact these authors comment that an 800 fold increase in serpentine accumulation occurs at 27°C on removal of 2,4-D (or replacement with NAA) than observed at 16°C in the presence of 2,4-D.

The data presented in Figure 1 suggests that a stepped temperature profile during the culture period, where cells are grown for a period at high temperature to maximise growth and then at a lower temperature to maximize product, may give higher alkaloid productivity than a constant temperature profile throughout the culture period. The data in Figure 1 was therefore fitted to the growth/product model described previously (Bailey et al., 1985). The results of this modeling, which will be described in detail elsewhere, predicted that for a 14 day fermentation a 21% increase in alkaloid yield could theoretically be obtained if cells were grown at 30°C for two days and then at 22°C for 12 days. The predictions made by this model are currently being tested experimentally.

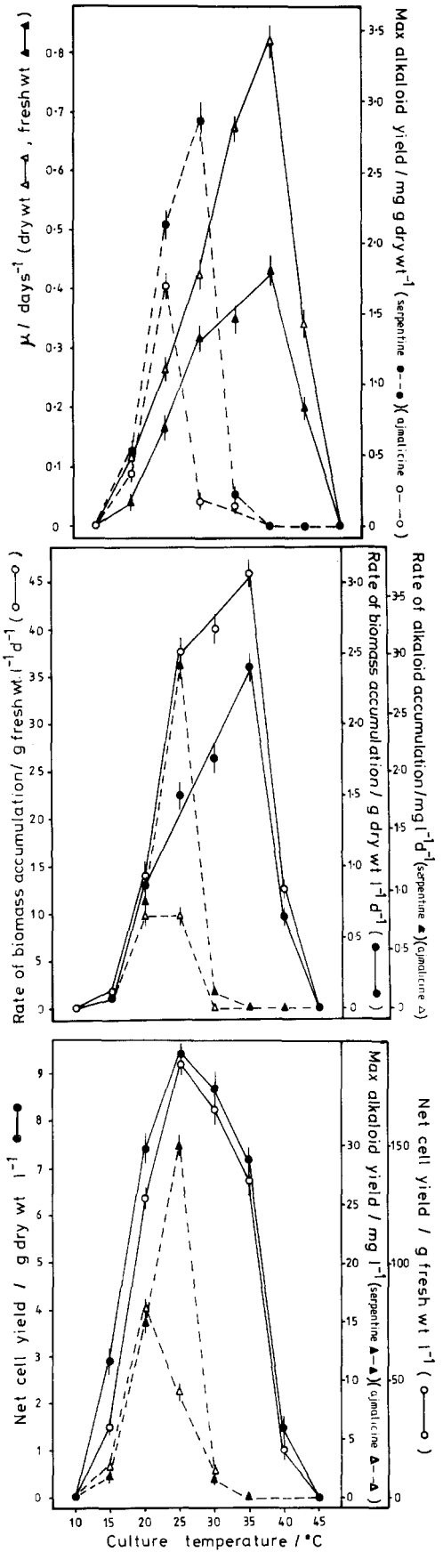
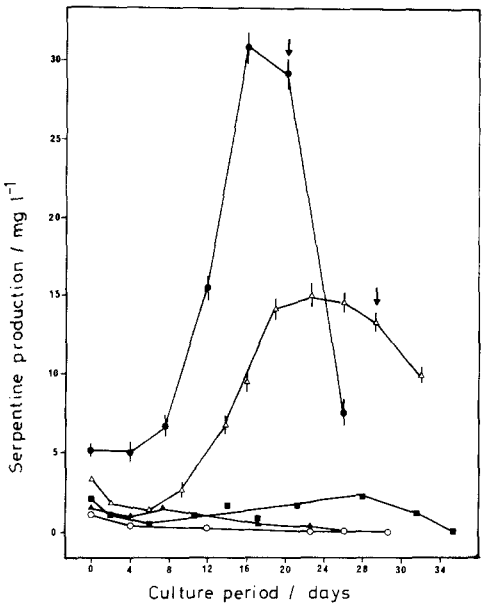
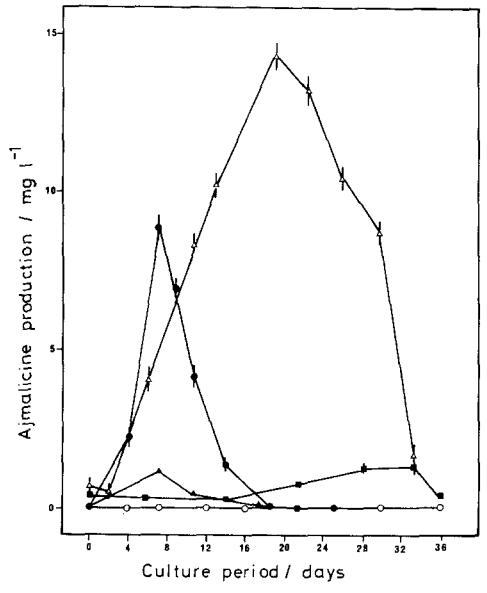


Figure 1: Effect of culture temperature on growth rate, biomass accumulation and alkaloid production in cell suspension cultures of Catharanthus roseus cell line C87 on M_3 medium.
Growth and alkaloid yields were determined from growth curves at each temperature. Points represent the mean ± SEM of three samples. All experiments were run in duplicate at different times.
Growth temperatures were maintained at the stated values ± 1°C.

Figure 2: Kinetics of serpentine and ajmalicine accumulation at various culture temperatures. 10°C (○), 15°C (■), 20°C (△), 25°C (●), 30° (▲). Mean ± SEM (n=3). Arrows indicate fall in cell viability.





Acknowledgements

The author wishes to thank Professor M.W. Fowler for his support of this work, the Wolfson Foundation for financial support and Mrs. S.E. Beet and Mrs. H. Woodhead for assistance with cell culture and alkaloid analysis.

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